Prospective Evaluation of a Semiquantitative Dip Slide Method Compared With Quantitative Bacterial Cultures of BAL Fluid*

Rudolf Speich, MD, FCCP; Jürg Wüst, PhD; Thomas Hess, MD; Fritz H. Kayser, MD; and Erich W. Russi, MD, FCCP

Background: Quantitative bacteriologic workup of BAL fluid (BALF) has evolved as a sensitive and specific technique for the diagnosis of bacterial pneumonia. Conventional quantitative cultures are expensive, time-consuming, and often unavailable on a 24-h basis. Therefore, we evaluated a dip slide method for the semiquantitative measurement of bacterial cultures in BALF specimens and compared the results with those from conventional quantitative cultures.

Methods: Fifty BALF specimens from 45 patients with suspected pulmonary infection were examined prospectively with both methods. We compared the microbiologic results of conventional quantitative cultures with those of the dip slide method that is commercially available for blood cultures. Cost-effectiveness analysis of both methods was performed.

Results: In 37 BALF specimens, 64 bacterial strains were detected with both techniques. The dip slide method and conventional cultures showed a high correlation with respect to the colony counts of the individual organisms per milliliter BALF (r=0.935; p=0.0001) and the sum of colony counts in individual patients (r=0.947; p=0.0001). Although five strains were not detected by the dip slide technique, the diagnostic accuracy was not influenced. In 13 BALF samples, there was no growth of bacteria with both techniques. While the diagnostic yield of both methods was similar, the dip slide technique was 44 to 66% less expensive than conventional cultures.

Conclusions: The examination of BALF with a dip slide method is highly comparable to conventional quantitative culture techniques, less expensive, and can be used independently of a specialized microbiology laboratory on a 24-h basis.

Key words: bacterial pneumonia; bronchoalveolar lavage; diagnosis

Abbreviations: BALF= BAL fluid; cfi= colony forming units

The diagnosis of bacterial pneumonia remains difficult. In one third of the patients ventilated for ARDS, pneumonia is misdiagnosed when compared with the results of histologic examination of lung tissue. Therefore, a correct diagnosis of bacterial pneumonia in this setting appears necessary to prevent undertreatment or overtreatment of many patients. Furthermore, antimicrobial therapy is remarkably simplified when the responsible pathogen is accurately defined, allowing a treatment with the most narrow and effective regimen of antibiotics avoiding toxicity and extensive costs. It has been shown by several investigators that analysis of BAL fluid (BALF) may be an important diagnostic tool for the diagnosis of bacterial pneumonias. Unfortunately, owing to contamination of the bronchoscope suction channel by oropharyngeal bacteria, the interpretation of nonquantitated bacteriologic cultures of BALF is not reliable. In their

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landmark studies, Kahn and Jones⁴ and Thorpe et al⁶ have shown that quantitative bacterial cultures of BALF are valuable for the diagnosis of bacterial pneumonia in nonventilated patients, distinguishing contamination and/or colonization from infection at 10³ colony forming units (cfu) per milliliter of BALF as a cut-off point. Sensitivity for detecting bacterial pneumonia ranged from 87 to 100%, and specificity ranged from 70 to 100%. Further studies in ventilated patients have suggested that quantitative bacterial cultures of BALF may enable the physician to distinguish between infection and colonization.³,⁵,⁷ The early recognition of bacterial pneumonia and its appropriate antimicrobial therapy are crucial for reducing the mortality of these patients and minimizing the emergence of resistant microorganisms.⁸ However, the widespread clinical application of a quantitative bacteriologic workup of BALF is limited by the fact that this procedure is expensive and relies on specialized microbiology laboratories. Furthermore, in most hospitals, facilities for this type of microbiologic procedure are not readily available on a 24-h basis.

The goal of this study was the evaluation of a simple semiquantitative dip slide method applied to BALF specimens. We used commercially available culture slides⁹ and compared the microbiologic results with those of conventional quantitative bacterial cultures (serial dilution method).

**Materials and Methods**

We studied 45 patients (mean age, 45 years; range, 18 to 77 years; 7 women, 38 men) undergoing BAL because of suspected pulmonary infections. Fourteen patients were intubated and mechanically ventilated. Twenty-four were immunosuppressed (11 HIV infected). Eight patients had been treated with antibiotics before BAL was performed. In five patients, two different areas were lavaged sequentially during the same examination. For comparison of the clinical diagnosis with the BALF results, only the BALF samples from the radiographically most involved area were considered.

The fiberoptic bronchoscope was inserted through a nostril in the nonintubated patients, and through an endotracheal tube via a sterile swivel adapter in the ventilated patients. Nonintubated patients received 0.5 mg atropine sulfate and 7.5 to 15 mg hydrocodone subcutaneously 15 min before bronchoscopy. IV administered flunitrazepam was used for additional sedation. The patients received 5 mL nebulized 4% lidocaine followed by 10 mL of 1% lidocaine injected through the bronchoscope onto the vocal cords. Intubated patients received midazolam and morphine sulfate IV before and during the procedure. The tip of the bronchoscope was gently wedged into the subsegmental bronchi leading to the area of radiographic abnormality. Four 50-mL aliquots of sterile isotonic saline solution were injected and then gently hand aspirated with a syringe. Fluid recovery ranged from 40 to 80%. The BALF was filtered through a double layer of sterile surgical gauze and poured
### Table 1—Results of the Conventional Technique and the Dip Slide Method in Patients With Clinically Probable Bacterial Pneumonia

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mechanical Ventilation</th>
<th>Immunosuppression*</th>
<th>Receiving Antibiotics</th>
<th>Conventional Cultures, cfu/mL</th>
<th>Log₁₀ of Sum cfu/mL</th>
<th>Dip Slide Method, cfu/mL</th>
<th>Log₁₀ of Sum cfu/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No (Alcoholism)</td>
<td>No</td>
<td>Man factor</td>
<td>6x10⁶ normal oral flora</td>
<td>2.8</td>
<td>4x10⁶ normal oral flora</td>
<td>2.6</td>
</tr>
<tr>
<td>2</td>
<td>No (Alcoholism)</td>
<td>No</td>
<td>1.2x10⁶ normal oral flora</td>
<td>4.1</td>
<td></td>
<td>1.1x10⁶ normal oral flora</td>
<td>4.0</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>No</td>
<td>6x10⁶ viridans streptococci</td>
<td>4.4</td>
<td></td>
<td>5x10⁶ normal oral flora</td>
<td>4.7</td>
</tr>
<tr>
<td>4</td>
<td>No HIV+</td>
<td>No</td>
<td>2.2x10⁴ H influenza</td>
<td>4.7</td>
<td>7x10⁴ H influenza</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>No HIV+</td>
<td>No</td>
<td>6x10⁴ S pneumoniae</td>
<td>4.8</td>
<td>1x10⁴ S pneumoniae</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Yes¹</td>
<td>No</td>
<td>5x10⁴ S pneumoniae</td>
<td>5.7</td>
<td>1x10⁴ S pneumoniae</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>No (Alcoholism)</td>
<td>No</td>
<td>1.6x10⁵ H influenza</td>
<td>5.4</td>
<td>1x10⁵ H influenza</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Yes¹ HIV+</td>
<td>No</td>
<td>1x10⁵ S milleri</td>
<td>5.1</td>
<td>1x10⁵ S milleri</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>No HIV+</td>
<td>No</td>
<td>6.5x10⁴ S pneumoniae</td>
<td>5.3</td>
<td>2x10⁴ S pneumoniae</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Yes¹</td>
<td>RTPL</td>
<td>4.5x10⁴ H influenza</td>
<td>7.6</td>
<td>1x10⁴ E coli</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>No HIV+</td>
<td>No</td>
<td>1.6x10⁵ H influenza</td>
<td>5.8</td>
<td>1x10⁵ H influenza</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>No HIV+</td>
<td>No</td>
<td>1.1x10⁶ viridans streptococci</td>
<td>5.0</td>
<td>5x10⁶ viridans streptococci</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>No HIV+</td>
<td>No</td>
<td>Legionella sp (BCYE medium)³</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

*HIV+=HIV infection; RTPL=renal transplant recipient.

¹Normal oral flora=2 or more of the following bacteria combined: viridans streptococci, Neisseria sp, coryneforms, coagulase-negative staphylococci.

²Mechanical ventilation because respiratory failure due to pneumonia; bronchoscopy and BAL within 48 h after intubation.

³BCYE medium-buffered charcoal yeast extract medium.

The diagnosis of a “probable bacterial pneumonia” was made in the presence of all of the following clinical criteria: (1) fever greater than 38.5°C, and/or purulence of tracheobronchial secretions, and/or leukocytosis greater than 10,000/µL or greater than 15% band forms; (2) a new or progressive localized radiographic infiltrate; and (3) improvement after appropriate antimicrobial therapy. An alternative diagnosis was established for all non-pneumonic infiltrates.

The costs of both methods were calculated on the basis of 100 examinations and assuming different prevalences of pneumonia and different sensitivities and specificities of BALF quantitative cultures for the diagnosis of bacterial pneumonia. In Switzerland, the charge for a conventional culture of BALF using serial dilutions is $54 if there is no detectable bacterial growth, and $96 if identification of bacterial strains is necessary. We assumed that only bacterial strains growing in significant cfu/mL are submitted to further identification. One dip slide costs $4, and the further microbiologic workup of slides with significant bacterial growth amounts to $89. We assumed that slides with nonsignificant growth of bacteria are thrown away.

Calculation of costs (Table 2) were done according to the following example, which assumes a prevalence of bacterial pneumonia of 20% and a diagnostic sensitivity and specificity of the BALF procedure of 90% each. Hence, the costs are as follows:

(A) Conventional cultures amount to:

- 18 true-positives and 26x$96 = $2,496
- 8 false-positives: 74x$54 = $3,996
- all negatives: $6,492, approximately $64 per patient

(B) Dip slide method amounts to:

- 1425

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Table 2—Comparison of Costs for Both Methods

<table>
<thead>
<tr>
<th>Conventional Cultures</th>
<th>Dip Slide Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Costs per Patient in Dollars*</td>
<td>Mean Costs per Patient in Dollars (% of Costs of Conventional Cultures)</td>
</tr>
<tr>
<td>Prevalence of pneumonia 20%</td>
<td>Prevalence of pneumonia 20%</td>
</tr>
<tr>
<td>Sensitivity and specificity 90%</td>
<td>65</td>
</tr>
<tr>
<td>Sensitivity and specificity 80%</td>
<td>67</td>
</tr>
<tr>
<td>Prevalence of pneumonia 40%</td>
<td>Prevalence of pneumonia 40%</td>
</tr>
<tr>
<td>Sensitivity and specificity 90%</td>
<td>72</td>
</tr>
<tr>
<td>Sensitivity and specificity 80%</td>
<td>72</td>
</tr>
<tr>
<td>Prevalence of pneumonia 60%</td>
<td>Prevalence of pneumonia 60%</td>
</tr>
<tr>
<td>Sensitivity and specificity 90%</td>
<td>78</td>
</tr>
<tr>
<td>Sensitivity and specificity 80%</td>
<td>78</td>
</tr>
</tbody>
</table>

*For explanation of calculations see Materials and Methods section.

7 through 9) all suffered from pneumonia and had significant growth of other bacterial strains on the dip slide. Therefore, the diagnostic accuracy of the dip slide technique was not influenced by the absence of growth in these five instances.

In 13 patients (Table 1), the diagnosis of “probable bacterial pneumonia” was made by clinical criteria. None of the remaining 32 patients had or developed bacterial pneumonia, and an alternative diagnosis was established by other means in all cases: bronchitis with normal chest radiograph (seven patients), mucous plugging in mechanically ventilated patients with normalization of the chest radiographic changes after removal of the plugs (seven), pulmonary involvement by neoplasia (four), ARDS without improvement of radiographic findings after antibiotic therapy (three), Pneumocystis carinii pneumonia without localized infiltrates (three), drug-induced pneumonitis with improvement after discontinuation of treatment with of the implicated drug (two), systemic lupus erythematosus with alveolar hemorrhage (one), cytomegalovirus pneumonia without localized infiltrates (one), invasive aspergillosis (one), bronchiolitis obliterans organizing pneumonia (one), HIV-associated nonspecific interstitial pneumonitis (one), and pleural effusion without pulmonary infiltrates after drainage (one).

In 7 of the 13 patients with probable bacterial pneumonia (Table 1, cases 6 through 12) the sum of cfu/mL BALF detected by conventional cultures was 10^5 or greater. In one patient (case 13), Legionella sp grew on special cultures (buffered charcoal yeast extract). Thus, conventional culture technique correctly identified 8 of 13 cases (62%) with clinically probable bacterial pneumonia.

With the dip slide technique, the sum of cfu/mL BALF was above the cutoff level in 9 of the 13 patients (69%) with probable bacterial pneumonia. Legionella sp did not grow on the dip slide.

The “specificity” regarding the clinical diagnosis of probable bacterial pneumonia was 100% (32/32) with both techniques. The “diagnostic accuracy” of the
conventional technique and the dip slide method was 90% (40/45) and 91% (41/45), respectively.

The comparison of the costs of both methods assuming different prevalences of bacterial pneumonia and different diagnostic sensitivities and specificities of BALF regarding the diagnosis of pneumonia is shown in Table 2. The dip slide method was 44 to 66% less expensive than the conventional culture technique.

**Discussion**

This prospective study shows that the results of the semiquantitative dip slide method used for the bacteriologic analysis of BALF are in good agreement with those of the conventional quantitative bacterial culture technique. The correlation of the cfu/mL BALF for individual bacterial strains and the sum of cfu/mL per BALF specimen determined with both methods was excellent (Fig 2).

Three organisms at high concentrations (>10^5 cfu/mL BALF) were not detectable by the dip slide method. Since this occurred in three patients with growth of a significant number (>10^5 cfu/mL BALF) of other bacterial strains, we suspect that the identification of these organisms was hindered by the concomitant overgrowth of a large number of other microorganisms. Although missing these three organisms by the dip slide method might have influenced the choice of the antibiotic regimen, in no instance was the diagnosis of pneumonia missed because of the growth of other bacterial strains at significant colony counts.

In one patient (case 13), the diagnosis of pneumonia due to Legionella sp could not be established by the dip slide technique. However, also in the microbiology laboratory, the detection of this organism requires the processing of BALF on special culture media.

The diagnostic impact of BAL for the detection of bacterial pneumonia was not addressed in this study because diagnosis of pneumonia solely relied on clinical criteria and not on generally accepted features such as histologic examination of lung tissue, positive blood or pleural cultures, or pulmonary abscesses with positive needle aspirate culture.13 Nevertheless, we are quite confident with our clinical diagnoses. For example, three of our four mechanically ventilated patients with pneumonia actually had to be intubated and ventilated because of respiratory failure due to pneumonia, and their clinical course with complete resolution after antibiotic therapy makes bacterial pneumonia the most likely diagnosis. Furthermore, in all the patients not classified as suffering from pneumonia on clinical grounds, an alternative diagnosis could be established. In summary, with regard to the clinical diagnosis of probable bacterial pneumonia in our study population, the diagnostic yield of both methods was comparable. The conditions of 62% and 69% of patients with clinically probable bacterial pneumonia were detected by the conventional culture technique and the dip slide method, respectively. This relatively low sensitivity of BAL for detecting clinically probable bacterial pneumonia in our study compared with others4,7 may partially be due to false-negative results of our aerobic culture technique in patient 1 and 2, who suffered from aspiration pneumonia with lung abscess formation most likely due to anaerobic bacteria. However, this problem might be circumvented by doing cultures (conventional or dip slide) in an anaerobic jar.14 Furthermore, the sensitivity of BAL for the diagnosis of bacterial pneumonia may be reduced by previous antibiotic therapy.15,16 Therefore, it is noteworthy that 8 of our 45 patients had received antibiotics before BAL.

Our study does not imply that BAL is a widely accepted and practiced method for the diagnosis of bacterial pneumonia. In fact, there is still much controversy about this subject.15,17,18 While some authors
prefer the protected specimen brush, others recommend the examination of endotracheal aspirates. The advantage of the protected specimen brush is the possibility of an uncontaminated recovery of potentially infected alveolar fluid. This, however, can also be accomplished by BAL using a protected, balloon-tipped BAL catheter. Despite the ongoing debate about the most appropriate technique for the diagnosis of pneumonia, most experts agree on the fact that only quantitative microbiological workup of specimens from the lower respiratory tract will help to differentiate among colonization, contamination, and infection. So far, we have shown by the present study that a semiquantitative workup of BALF recovered from the respiratory tract can be performed reliably and independently of a microbiology laboratory by using the dip slide method. It is conceivable that the dip slide method can also be applied to other respiratory specimens such as endotracheal aspirates, specimens from protected specimen brush, or BALF retrieved by a protected, balloon-tipped catheter.

Another obstacle for a more widespread use of BAL for the diagnosis of bacterial pneumonia is the lack of agreement on the cutoff level of cfu/mL to separate colonization or contamination from infection. While a consensus conference suggested $10^4$ cfu/mL BALF as an appropriate cutoff, cutoff levels of $10^3$ to $10^5$ cfu/mL are used by different authors. The dip slide method, however, can be used with any chosen cutoff level since its results are comparable to the conventional technique over the entire range of concentrations of bacteria (Fig 2).

The dip slide method is markedly less expensive than the conventional culture technique using the serial dilution method and can be used even in the physician’s office. While slides with absent or nonsignificant bacterial growth may be discarded, those with more than $10^4$ to $10^5$ cfu/mL, depending on the chosen cutoff level, can be sent to the microbiology laboratory for further workup and susceptibility testing. Using this procedure and different prevalences of bacterial pneumonia, cost-effectiveness analysis revealed that the dip slide method was 44 to 66% less expensive than the conventional technique.

One might argue that our patient population was heterogeneous, comprising immunocompetent and immunosuppressed as well as ventilated and nonventilated patients. However, the goal of this study was not the assessment of BAL as a diagnostic tool but the validation of the dip slide method in comparison to the conventional serial dilution technique. Therefore, it was important to test BALF samples from different patient populations with expectedly different colony counts.

In conclusion, our study shows that the dip slide technique can be used as a reliable and cheap tool to measure bacterial growth in BALF specimens. This semiquantitative technique is comparable to the conventional serial dilution method. In contrast to conventional quantitative cultures, however, the dip slide technique can be performed on a 24-h basis independently of a specialized microbiology laboratory. If no growth or a low number of bacterial colonies is found, the dip slide may be discarded to avoid further time-consuming and costly workup. However, a detailed microbiologic workup with identification of the microorganisms and susceptibility testing is not precluded and can be performed at a convenient time.

References

17. Chastre J, Fagon JY. Invasive diagnostic testing should be
routinely used to manage ventilated patients with suspected pneumonia. Am J Respir Crit Care Med 1994; 150:570-74
18 Niederman MS, Torres A, Summer W. Invasive diagnostic testing is not needed routinely to manage suspected ventilator-associated pneumonia. Am J Respir Crit Care Med 1994; 150: 565-69